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School of Pharmaceutical Sciences of Ribeirão Preto

and

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Centre for Forensic Science

Sweat as an alternative sample in doping control

Dayanne Cristiane Mozaner Bordin

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"There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle".

Albert Einstein

ABSTRACT

Bordin, Dayanne Cristiane Mozaner. **Sweat as an alternative sample in doping control.** 2019. 149p. Thesis Doctor Degree. School of Pharmaceutical Sciences- USP and Centre of Forensic Sciences-UTS. 2019.

Sport has become a major industry of invaluable awards and major investments. To overcome opponents, many athletes resort to illegal means to improve their performance, as the World Antidoping Agency (WADA) prohibits drug use in sport. In addition to the ethical aspects, drug use can cause serious damage to an athletes' health by directly influencing physiological capacity and removing physical and psychological barriers, and their health damage may be irreversible. Urine is the standard matrix used in doping control; however, these analyses can also be performed on alternative biological samples; such as sweat. Toxicological analysis in sweat samples present some advantages over urine, such as less chance of sample tampering, greater detection windows, non-invasive collection, and the possibility of finding the parent drugs. The use of sweat as an alternative sample also allows obtaining a history of drug abuse.

The identification of doping cases has been an important area of research and development in recent years due to the continued increase in the number of prohibited substances. For the detection of prohibited substances, sophisticated analytical instruments such as liquid (LC), gas (GC) chromatography coupled to mass spectrometry and capillary electrophoresis (CE) are generally used. This thesis focused on the development of methods for evaluation of sweat as an alternative biological matrix for doping control, using different methods for sample preparation and detection to overcome its limitations. In addition, an outline of the profile of drugs used for sports practice and the acute effects caused in the organism of abusers were investigated.

A survey was applied to the volunteers to evaluate the adverse effects of androgenic anabolic steroids (AAS), dietary supplements (DS) and multiple drug use. In parallel, the volunteers hematological and biochemical parameters were measured. Through toxicological urine analysis, self-reporting and the parameters measured within the study revealed that the use of doping agents and drugs are subject to different factors, which are normally guided by the type of physical activity or aesthetic appearance. This abuse can increase the chances of health problems causing synergistic side effects, increasing the risk to develop diseases. We developed a method to determine the presence of 13 amphetamines and cocaine related

substances and their metabolites in sweat and urine using disposable pipette extraction (DPX) tips and GC-MS. The validated method was used to analyze 40 urine and sweat samples whose athletes self-reported the use of drugs and/or stimulants. It was verified that all consumed drugs and metabolites detected in urine were also present in sweat samples indicating that sweat was a viable matrix to perform doping tests.

We also developed a screening alternative method for stimulants detection in sweat samples using a microchip capillary electrophoresis instrument (Agilent Bionalyzer). Although functioning, the method was not sensitive enough to detect the low concentrations of drugs and metabolites present in sweat samples.

A one step fully automatized derivatisation and headspace (HS) SPME extraction method followed by GC-MS was developed for the analysis for amphetamine-type drugs and cocaine. The HS-SPME/GC-MS method was used to detect concentrations between 0.1 to 1 ng/mL of the target analytes without any additional sample preparation, suitable for routine analysis of drug traces in biological samples, such as urine and sweat.

Keywords: sport, doping, alternative biological matrix, sweat stimulants, urine, and analytical methods.

RESUMO

Bordin, Dayanne Cristiane Mozaner. **Suor como matriz alternativa no controle do doping.** 2019. 149p. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2019.

O esporte tornou-se uma indústria de valiosos prêmios e grandes investimentos. Na tentativa de vencer adversários, muitos atletas recorrem a meios ilegais para melhorar seu desempenho. A Agência Mundial Antidoping (WADA) proíbe o uso de drogas no esporte. Além dos aspectos éticos, o uso de drogas pode causar sérios danos à saúde de um atleta, influenciando diretamente a capacidade fisiológica e removendo barreiras físicas e psicológicas, e seus danos à saúde podem ser irreversíveis. A urina é a matriz padrão utilizada no controle de doping. No entanto, essas análises também podem ser realizadas em amostras biológicas alternativas; como o suor. A análise toxicológica em amostras de suor apresenta algumas vantagens em relação à urina, como menor chance de adulteração de amostras, maior janela de detecção, coleta não invasiva, além da possibilidade de encontrar principalmente as drogas consumidas. O uso de suor como amostra alternativa também permite obter um histórico de exposição ao abuso de drogas. A identificação de casos de doping tem sido uma área importante de pesquisa e desenvolvimento nos últimos anos devido ao aumento contínuo do número de substâncias proibidas. Para a detecção de substâncias proibidas, geralmente são utilizados instrumentos analíticos sofisticados, tais como cromatografia líquida (LC), cromatografia gasosa (GC) acoplados à espectrometria de massas e eletroforese capilar (CE). Esta tese centrou-se no desenvolvimento de métodos para avaliação do suor como uma matriz biológica alternativa para o controle de doping, utilizando diferentes métodos de preparação e detecção de amostras para superar suas limitações. Paralelamente, foi realizado um esboço do perfil das drogas utilizadas para prática esportiva e os efeitos agudos causados no organismo dos usuários. Foi aplicada uma pesquisa aos voluntários para avaliar os efeitos adversos dos esteroides anabolizantes androgênicos (AAS), suplementos alimentares (DS) e uso múltiplo de drogas. Em paralelo, foram realizadas medidas de parâmetros hematológicos e bioquímicos dos voluntários. Através de análises toxicológicas em urina, o auto-relato e a medição de parâmetros, o estudo revelou que o uso de agentes dopantes e drogas recreativas são submetidos a diferentes fatores, que são normalmente guiados pelo tipo de atividade física ou aparência estética. Seu uso pode aumentar as chances de problemas de saúde causando efeitos colaterais sinérgicos, aumentando o risco de desenvolver doenças. Desenvolvemos um

método para determinar a presença de 13 anfetaminas e substâncias relacionadas à cocaína e seus metabolitos em suor e urina usando Pipetas de Extração descartáveis (DPX) e GC-MS. O método validado foi utilizado para analisar 40 amostras de urina e suor cujos atletas auto-relataram o uso de drogas. Verificou-se que todas as drogas consumidas e seus metabólitos encontradas na urina também estavam presentes em amostras de suor indicando que o suor é uma matriz viável para realizar testes de doping. Também desenvolvemos um método alternativo de triagem para detecção de estimulantes em amostras de suor usando um instrumento de eletroforese capilar de microchip (Bionalyzer). Embora funcionasse, o método não era suficientemente sensível para detectar baixas concentrações de drogas e metabólitos presentes em amostras de suor. Foi desenvolvido um método de extração por headspace (HS) e derivatização totalmente automatizado de apenas um passo seguido por analises em GC-MS para determinação de drogas tipo anfetaminas e cocaína. O método HS-SPME/GC-MS foi utilizado para detectar concentrações entre 0,1 a 1 ng/mL dos analitos sem qualquer preparação adicional de amostra, apresentando potencial aplicação para análise de rotina de traços de drogas em amostras biológicas, como urina e suor.

Palavras-chave: esporte, doping, matriz biológica alternativa, estimulantes, agentes anabolicos esteroides, suor, urina e métodos analíticos.

CERTIFICATE OF AUTHORSHIP AND ORIGINALITY

I, Dayanne Cristiane Mozaner Bordin, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis has also been submitted for qualification at University of São Paulo as it is the result of a research candidature conducted as part of a collaborative Doctoral degree.

This document has not been submitted for qualifications at any other academic institution. This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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LIST OF CONFERENCES

The research conducted during this project was presented at several international conferences listed below.

Year	Conference	Presentation
2014	52rd The International Association of Forensic Toxicologists (TIAFT), Buenos Aires, Argentina	A rapid assay for determination of MDMA, MDA, MDEA, Methamphetamine and Amphetamine in sweat using Disposable Pipette Extraction (DPX) and GC-MS
2015	53rd The International Association of Forensic Toxicologists (TIAFT), Firenze, Italy	Side effects and trends in the consumption of anabolic-androgenic steroids (AASs) and psychotropic drugs in bodybuilders
2015	9th Congress of Toxicology in Developing Countries XIX Congresso Brasileiro de Toxicologia, Natal, Brazil	Side effects in the consumption of Anabolic Androgenic Steroids And Dietary Supplements
2016	54rd The International Association of Forensic Toxicologists (TIAFT), Brisbane, Australia	A new approach for the analysis of stimulants in sweat using a disposable pipette extraction (DPX) and GC-MS
2016	International Symposium on Advances in Separation Science (ASASS), Hobart, Tasmania	Application of screening method for stimulants in sweat samples and urine using a modified technique DPX and GC-MS
2017	Forensic and Clinical Association Meeting (FACTA), Melbourne, Australia	One step automatized analysis of drug stimulants using HS-SPME/GC-MS

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ABBREVIATIONS

WADA	World Antidoping Agency
LC	Liquid Chromatography
GC	Gas Chromatography
MS	Mass Spectrometry
CE	Capillary Electrophoresis
DPX	Disposable Pipette Extraction Tips
AAS	Anabolic Androgenic Steroids
DS	Dietary Supplements
EI	Electro Ionization
HS	Headspace
SPME	Solid Phase Micro Extraction
IOC	International Olympic Committee
S	Substances
M	Methods
HIF	Hypoxia-inducible factor stabilizers
CG	Chorionic Gonadotrophin
GH	Growth Hormone
SERM	Selective estrogen receptor modulators
mL	Millilitre
CNS	Central Nervous System
DAT	Dopamine
NOR	Noradrenaline
SER	Serotonin
MA	Methamphetamine
MDA	3,4-methylenedioxyamphetamine

MDMA	3,4-methylenedioxymethamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
BE	Benzoylecgonine
EME	Methylecgonine ester
DHEA	Dehydroepiandrosterone
cAMP	Cyclic adenosine monophosphate
RNA	Ribonucleic
LH	Luteinizing Hormone
FSH	Follicle-stimulating Hormone
T4	Thyroxine
T3	Triiodothyronine
IRMS	Isotope Ratio Mass Spectrometry
LLE	Liquid–Liquid Extraction
SPE	Solid-Phase Extraction
RP	Reversed-Phase sorbent
CX	Cation Exchanger sorbent
WAX	Anion Exchanger sorbent
PDMS	Polydimethylsiloxane
PA	Polyacrylate
CAR	Carbowax
CI	Chemical Ionisation
QMS	Single Quadrupole Mass Spectrometer
S/N	Signal-to-noise
QQQ	Triple Quadrupole
ME	Microchip electrophoresis
EOF	Electroosmotic flow

MEKC	Micellar Electrokinetic Capillary Chromatography
UV	ultra-violet
CID	Collision-Induced Dissociation
PES	Performance Enhancing Substances
THCCOOH	11-nor-9-carboxy-9-tetrahydrocannabinol
d	Deuterium
mg	Milligram
C	Celsius
°	Degrees
MSTFA	N-methyl-N-(trimethylsilyl) trifluoroacetamide
µg	Microgram
IS	Internal Standard
M	Molar
rpm	Rotation per minute
v	Volume
mm	Millimetre
m	Metre
µm	Micrometre
SIM	Selected Ion-Monitoring
m/z	Mass-to-charge ratio
min	Minutes
LOD	Limit of Detection
LOQ	Limit of Quantification
PC	Positive Control
THC	Cannabis
BCAA	Amino acids

MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
GFR	Glomerular Filtration Rate
dL	Decilitre
L	Litre
CKD	Chronic kidney disease
GGT	Gama-Glutamyl Transferase
GOT	Glutamico-Oxalacetic Transaminase
GPT	Glutamic-Pyruvic Transaminase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
VLDL	Very Low-density lipoprotein
MPT	Mephentermine
AMP	Amphetamine
AMP D11	Amphetamine D11
METAMP	Metamphetamine
AMPHEP	Amphepramone
MDMA	3,4-Methylenedioxymethamphetamine
MDMA D5	3,4-Methylenedioxymethamphetamine D5
MDA	3,4-Methylendioxyamphetamine
MDEA	3,4-Methylenedioxyethylamphetamine
MPH	Methylphenidate
FENP	Fenproporex
COC	Cocaine
COC D3	Cocaine D3

BE	Benzoylecgonine
COE	Cocaethylene
MEPHE	Mephedrone
SWGTOX	Scientific Working Group Toxicology guidelines
R^2	Coefficient of determination
QC	Quality control
ng	Nanogram
LOC	Lab-on-a-chip
mmol	Millimol
LED	Fluorescence detection
FITC	Fluoresceine isothiocyanate
mM	Millimol
kV	Kilovolts
ppt	Parts per trillion
cEF	ethyl chloroformate